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(71) Applicant: CHILDREN'S MEDICAL CENTER CORPORATION [US/US]; 55 Shattuck Street, Boston, MA 02115 (US).

(72) Inventors: BERDE, Charles, B.; 14 Doran Road, Brookline, MA 02146 (US). LANGER, Robert, S.; 77 Lombard Street, Newton, MA 02139 (US).

(74) Agent: PABST, Patrea, L.; Arnall Golden & Gregory, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).

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(57) Abstract

It has been discovered that glucocorticoids prolong nerve blockage produced by release of local anesthetic agents from local delivery systems such as microspheres. The degree of prolongation is proportional to the strength of the glucocorticoid. Preferred anesthetics are local anesthetics that induce local pain relief or numbness, especially bupivacaine, dibucaine, etidocaine, and tetracaine, most preferably the free base of the local anesthetic. Preferred glucocorticoids are dexamethasone, methylprednisolone, betamethasone, and hydrocortisone. Synthetic polymers are preferred for formation of microparticles for release of the local anesthetic agent and glucocorticoid. The glucocorticoid is preferably incorporated into the polymeric matrix or administered with the microspheres. The microspheres are injected at the site where the anesthetic is to be released. This can be at the time of surgery, or irrigation or perfusion of a wound.

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PROLONGED NERVE BLOCKADE BY THE COMBINATION OF LOCAL ANESTHETICS AND GLUCOCORTICOIDS

Background of the Invention

This invention is generally in the field of anesthesiology and, in particular, the delivery of anesthetic agents in combination with glucocorticoids to provide prolong nerve blockade.

European Patent Application No. 93922174.3 by Children's Medical Center Corporation discloses biodegradable synthetic polymeric microparticles releasing local anesthetic over prolonged periods of time. Dexamethasone was included to avoid inflammation due to the polymer. Even though release of the local anesthetic was obtained over periods exceeding three days, with favorable release kinetics, prolonged nerve blockade did not correlate with release of anesthetic.

It is the object of this invention to provide an improved biodegradable controlled release delivery system which administers local anesthetic to provide more prolonged nerve blockade.

Summary of the Invention

It has been discovered that glucocorticoids prolong nerve blockade produced by release of local anesthetic agents from local delivery systems such as microspheres. The degree of prolongation is proportional to the strength of the glucocorticoid. Preferred anesthetics are local anesthetics that induce local pain relief or numbness, especially bupivacaine, dibucaine, etidocaine, and tetracaine, most preferably the free base of the local anesthetic. Preferred glucocorticoids are dexamethasone, methylprednisolone, betamethasone, and hydrocortisone. The local anesthetic is administered using a local delivery system, most preferably a synthetic polymer. Useful polymers

include polyanhydrides, poly(hydroxy acids), especially as polylactic acid-glycolic acid copolymers, and polyorthoesters, optionally containing a catalyst. Polylactic acid-glycolic acid copolymers are preferred. Local anesthetics are preferably incorporated into the polymer using a method that yields a uniform dispersion. The preferred form is injectable, for example, microspheres. The glucocorticoid is preferably incorporated into the polymeric matrix or administered with the microspheres. The type of anesthetic and glucocorticoid and the quantity are selected based on the known pharmaceutical properties of these compounds. As defined herein, prolonged means greater than one day.

The microspheres are injected at the site where the anesthetic is to be released. This can be at the time of surgery, or irrigation or perfusion of a wound.

Examples demonstrate prolongation of nerve blockade of the combination as compared with nerve blockade in the absence of the glucocorticoid. Other types of steroids and antiinflammatories did not extend release, nor was prolonged nerve blockade shown *in vitro*.

Brief Description of the Drawings

Figure 1 is a graph of percent cumulative release versus time (days) for release of bipivacaine from microspheres formed of polylactic acid-glycolic acid (PLGA), 75:25, with (squares) and without (triangles) 0.05% dexamethasone.

Figure 2 is a graph of the dose response curve (duration of latency, hr) for polylactic acid (PLA) (squares), PLGA 65:35 (circles), and PLGA 75:25 (triangles) microspheres loaded with bupivacaine and dexamethasone, administered at

doses of 50 mg of microspheres/kg rat. Error bars indicate standard errors.

Figures 3a and 3b are graphs of the duration of latency versus time (hours), determined by sensory testing using the modified hot plate test (Figure 3a) or by motor testing (Figure 3b) for 75% bupivacaine loaded PLGA 65:35 containing 0.05%, 0.005%, and 0% dexamethasone. Error bars indicate standard errors.

Figures 4a, b, c, and d, are graphs comparing the duration of latency versus time (hours), determined using the modified hot plate test for: 100 PLA microspheres loaded with 75% bupivacaine which contained 0.05% dexamethasone with corresponding microspheres which did not contain dexamethasone (Figure 4a); PLGA 75:25 microspheres loaded with 75% bupivacaine which contained 0.05% dexamethasone with corresponding microspheres which did not contain dexamethasone (Figure 4b); 65:35 PLGA microspheres loaded with 75% bupivacaine which contained 0.05% dexamethasone with corresponding microspheres which do not contain dexamethasone (Figure 4c); and 50:50 PLGA microspheres loaded with 75% bupivacaine which contained 0.05% dexamethasone with corresponding microspheres which do not contain dexamethasone (Figure 4d). Error bar indicate standard errors.

Figures 5a and b are graphs of the duration of sensory block (Figure 5a) and of the duration of motor block (Figure 5b) in hours after injection of bupivacaine loaded microspheres (circles), bupivacaine loaded microspheres with dexamethasone in the injection fluid (squares), and bupivacaine loaded microspheres with betamethasone in the injection fluid (triangles).

Figure 6 is a graph of sensory block over time after injection (hours) in rats of PLGA 65:35

microspheres containing bupivacaine and one of four glucocorticoids: betamethasone (0.8 mg/kg), dexamethasone (0.14 mg/kg), methylprednisolone (0.1 mg/kg), and hydrocortisone (1.25 mg/kg).

Figure 7 is graph of the percent cumulative release over time (days) showing *in vitro* release of all polymers containing dexamethasone, for PLA containing bupivacaine and dexamethasone (solid squares), PLGA 50:50 containing bupivacaine with dexamethasone (solid circles), PLGA 65:35 containing bupivacaine with (solid squares) and without dexamethasone (open squares), and PLGA 75:25 containing bupivacaine with (open squares) and without bupivacaine (solid squares).

Detailed Description of the Invention

Local delivery systems for the prolonged nerve blockade by a local anesthetic agent in combination with a glucocorticoid in a targeted area are provided. These systems can be used for the management of various forms of persistent pain, such as postoperative pain, sympathetically maintained pain, or certain forms of chronic pain such as the pain associated with many types of cancer. As used herein, "prolonged nerve blockade" means prolonged pain relief or numbness due to the combination of local anesthetic and glucocorticoid, as compared with pain relief or numbness obtained by administrtion of the local anesthetic in the absence of the glucocorticoid.

Anesthetics

The systems employ biodegradable polymer matrices which provide controlled release of local anesthetics. As used herein, the term "local anesthetic" means a drug which provides local numbness or pain relief. A number of different local anesthetics can be used, including dibucaine,

bupivacaine, ropivacaine, etidocaine, tetracaine, procaine, chlorocaine, prilocaine, mepivacaine, lidocaine, xylocaine, and mixtures thereof. The preferred anesthetic is bupivacaine or dibucaine, most preferably in the free base, alternatively in the form of a salt, for example, the hydrochloride, bromide, acetate, citrate, or sulfate. Bupivacaine is a particularly long acting and potent local anesthetic when incorporated into a polymer. Its other advantages include sufficient sensory anesthesia without significant motor blockage, lower toxicity, and wide availability.

The delivery systems can also be used to administer local anesthetics that produce modality-specific blockade, as reported by Schneider, et al., Anesthesiology, 74:270-281 (1991), or that possess physical-chemical attributes that make them more useful for sustained release then for single injection blockade, as reported by Masters, et al., Soc. Neurosci. Abstr., 18:200 (1992), the teachings of which are incorporated herein.

The anesthetic is incorporated into the polymer in a percent loading of 0.1% to 90% by weight, preferably 5% to 75% by weight. It is possible to tailor a system to deliver a specified loading and subsequent maintenance dose by manipulating the percent drug incorporated in the polymer and the shape of the matrix, in addition to the form of local anesthetic (free base versus salt) and the method of production. The amount of drug released per day increases proportionately with the percentage of drug incorporated into the matrix (for example, from 5 to 10 to 20%). In the preferred embodiment, polymer matrices with about 75% drug incorporated are utilized, although it is possible to incorporate substantially more drug,

depending on the drug, the method used for making and loading the delivery system, and the polymer.

Steroidal Antiinflammatories (Glucocorticoids)

Glucocorticoids that are useful to prolong *in vivo* release include glucocorticocoids such as dexamethasone, cortisone, hydrocortisone, prednisone, and others routinely administered orally or by injection. Other glucocorticoids include beclomethasone, dipropianate, betamethasone, flunisolide, methyl prednisone, para methasone, prednisolone, triamcinolome, alclometasone, amcinonide, clobetasol, fludrocortisone, diflurosone diacetate, fluocinolone acetonide, fluoromethalone, flurandrenolide, halcinonide, medrysone, and mometasone, and pharmaceutically acceptable salts and mixtures thereof. Useful loadings are from 0.01 to 30% by weight, preferably between 0.05 and 0.5%. The dosage must be low enough to avoid toxicity.

Delivery Systems

The local anesthetic is preferably delivered to the patient incorporated into a polymer in the form of microparticles, most preferably microspheres. Other forms of the polymers include microcapsules, slabs, beads, and pellets, which in some cases can also be formulated into a paste or suspension.

The delivery systems are most preferably formed of a synthetic biodegradable polymer, although other materials may also be used to formulated the delivery systems, including proteins, polysaccharides, and non-biodegradable synthetic polymers. It is most preferable that the polymer degrade *in vivo* over a period of less than a year, with at least 50% of the polymer degrading

within six months or less. Even more preferably, the polymer will degrade significantly within a month, with at least 50% of the polymer degrading into non-toxic residues which are removed by the body, and 100% of the anesthetic and glucocorticoid being released within a two week period. Polymers should also preferably degrade by hydrolysis by surface erosion, rather than by bulk erosion, so that release is not only sustained but also linear. Polymers which meet this criteria include some of the polyanhydrides, poly(hydroxy acids) such as co-polymers of lactic acid and glycolic acid wherein the weight ratio of lactic acid to glycolic acid is no more than 4:1 (i.e., 80% or less lactic acid to 20% or more glycolic acid by weight), and polyorthoesters containing a catalyst or degradation enhancing compound, for example, containing at least 1% by weight anhydride catalyst such as maleic anhydride. Other polymers include protein polymers such as gelatin and fibrin and polysaccharides such as hyaluronic acid. Polylactic acid is not useful since it takes at least one year to degrade *in vivo*.

The polymers should be biocompatible. Biocompatibility is enhanced by recrystallization of either the monomers forming the polymer and/or the polymer using standard techniques.

Although not as preferred, other local carrier or release systems can also be used, for example, the lecithin microdroplets or liposomes of Haynes, et al., Anesthesiology 63, 490-499 (1985), or the polymer-phospholipid microparticles of U.S. Patent No. 5,188,837 to Domb. As used herein, the term "polymer" refers interchangeably with the various carrier forms, including the lipid based carriers, unless otherwise specified.

Methods of Manufacture of Delivery Systems

Methods for manufacture of suitable delivery systems for administration of the local anesthetic in combination with glucocorticoid are known to those skilled in the art. The local anesthetic is incorporated, at least in part, into the delivery system. The glucocorticoid can be incorporated into all or a part of the delivery system(s), and/or administered adjacent to or with the delivery systems as a formulation.

As used herein, polymeric delivery systems include microparticles, slabs, beads, pastes, pellets, and suspensions. Microparticles, microspheres, and microcapsules are collectively referred to herein as "microspheres". Microspheres are used in the most preferred embodiment. The microspheres are preferably manufactured using methods for manufacture of microspheres which are well known and are typified in the following examples, most preferably a method that evenly disperses the anesthetic throughout the delivery system, such as solvent casting, spray drying or hot melt, rather than a method such as compression molding. A desired release profile can be achieved by using a mixture of microspheres formed of polymers having different release rates, for example, polymers releasing in one day, three days, and one week, so that linear release is achieved even when each polymer *per se* does not release linearly over the same time period. In the preferred embodiment for administration by injection, the microspheres have a diameter of between approximately 10 and 200 microns, more preferably between 20 and 120 microns.

Methods of Administration

In the preferred method of administration, the formulation consists of a suspension of microspheres which are administered by injection at

the site where pain relief is to be achieved. The microspheres may be injected through a trochar, or the pellets or slabs may be surgically placed adjacent to nerves, prior to surgery or following repair or washing of a wound. The microspheres can be administered alone when they include both the glucocorticoid and local anesthetic or in combination with a solution including a steroidal anti-inflammatory or other glucocorticoids in an amount effective to prolong nerve blockade by the anesthetic released from the microspheres. The suspensions, pastes, beads, and microparticles will typically include a pharmaceutically acceptable liquid carrier for administration to a patient, for example, sterile saline, sterile water, phosphate buffered saline, or other common carriers.

Potential applications include two to five day intercostal blockade for thoracotomy, or longer term intercostal blockade for thoracic post-therapeutic neuralgia, lumbar sympathetic blockade for reflex sympathetic dystrophy, or three-day ilioinguinal/iliohypogastric blockade for hernia repair.

The present invention is further described with reference to the following non-limiting examples.

The following methods were utilized in the *in vivo* studies on rats.

<u>Nerve Block Tests</u>

<u>Motor Block</u>

The rats were behaviorally tested for sensory and motor blockage in a quiet observation room at 24 ± 1°C. Testing was only performed in rats showing appropriate baseline hot plate latencies after at least one week of testing. In all testing conditions, the experimenter recording the behavior was unaware of the side containing the

local anesthetic. To assess motor block, a 4-point scale based on visual observation was devised: (1) normal appearance, (2) intact dorsiflexion of foot with an impaired ability to splay toes when elevated by the tail, (3) toes and foot remained plantar flexed with no splaying ability, and (4) loss of dorsiflexion, flexion of toes, and impairment of gait. For graphing clarity, partial motor block equals a score of 2 and dense motor block is a score of either 3 or 4.

Sensory Block

Sensory blockade was measured by the time required for each rat to withdraw its hind paw from a 56°C plate (IITC Life Science Instruments, Model 35-D, Woodland Hills, CA). They were tested between 10 am and 12 pm daily and allowed to adjust to their surroundings in a quiet room at 22 ± 1°C for at least 30 minutes before testing. The rats were held with a cloth gently wrapped above their waist to restrain the upper extremities and obstruct vision. The rats were positioned to stand with one hind paw on a hot plate and the other on a room temperature plate. With a computer data collection system (Apple IIe with a footpad switch), latency to withdraw each hind paw to the hot plate was recorded by alternating paws and allowing at least fifteen seconds of recovery between each measurement. If no withdrawal occurred from the hot plate within 15 seconds, the trial was terminated to prevent injury and the termination time was recorded. Testing ended after five measurements per side, the high and low points were disregarded, and the mean of the remaining three points was calculated for each side. Animals were handled in accordance with institutional, state and federal guidelines.

No rats were observed to have inflammation or blisters. Rats were tested for at least two weeks prior to surgery to achieve a consistent baseline latency, and testing continued for two weeks after surgery to confirm complete recovery from sensory blockade. Motor blockade was rated on a 4-point scale. Animals with a motor block of 4 had a clubbed hindpaw and usually dragged their affected leg when walking. Motor block 3 animals walked normally but had toes that failed to splay when the animal was lifted. Animals with motor block of 2 showed toes that splayed but not as fully as normal or motor block 1 animals.

Necropsy and Histology

Animals were sacrificed two weeks after implantation. Sections of sciatic nerve approximately 2-3 cm in length, adjacent and proximal to the implants, were preserved in 10% formalin solution (24 mM sodium phosphate, pH 7). Sections were then embedded in paraffin, stained with hematoxylin and eosin, and examined by light microscopy.

Plasma Analysis

Rats (250-275 g) anesthetized with ketamine-HCl (100 mg/ml at 1.5 ml/kg, i.p.) and xylazine (4 mg/ml at 4 mg/kg, i.p.), were implanted with a silastic catheter into the right jugular vein. Blood was withdrawn (0.5 cc) before implantation and at timed intervals after administration via the indwelling central venous cannulae. Plasma was extracted with an equal volume of HPLC grade methanol (Fischer Scientific, Pittsburgh, PA), centrifuged (10,000 x g) and the methanol phase filtered (0.2 μm nylon syringe type, Rainin, Woburn, MA) prior to HPLC analysis. The HPLC reliably quantified bupivacaine concentrations in the plasma methanol extraction phase down to 10

ng/ml. The bupivacaine standards used for blood plasma analyses were added to plasma aliquots prior to methanol extraction. The peak matching the standard bupivacaine peak's retention time was verified in plasma samples by doping with bupivacaine.

Statistics

Data were analyzed using linear regression tests, ANOVA, Chi Square tests and Wilcoxon rank-sum tests, where appropriate.

Example 1: Prolonged nerve blockade with steroidal antiinflammatories.

As demonstrated by the following study:

(1) Bupivacaine-polyester microspheres can be formulated with mechanical stability at very high percent drug loading, for example, up to 75% by weight.

(2) Bupivacaine-polyester microspheres with high percent loading have controlled release of drug, and do not produce rapid initial burst release of drug *in vitro* or *in vivo*.

Methods and Material

Abbreviations include PLGA, poly-lactic-glycolic acid; CH_2Cl_2, methylene chloride; PLAM, polymer local anesthetic matrices; dpm, disintegrations per minute; cpm, counts per minute; rpm, revolutions per minute.

The non-radioactive polymer microspheres used in this study were supplied by Medisorb, Cincinnati, OH. The PLGA 65:35 (Lot. No. S2170 Si177, Mw 130,000) was supplied by Medisorb, Cincinnati, OH. Tritium labeled dexamethasone was obtained from Amersham (specific activity 9.24 x 10^{10} dpm/μmole). Bupivacaine free base was supplied by Purdue Frederick (Lot No. 32931) and dexamethasone was supplied by Sigma (Lot No. 34H0502). Trisma base was supplied by Sigma (Lot No. 64H5732). Dulbecco's phosphate-buffered saline

was supplied by Gibco, Maryland (Lot No. 14N5447). (KCL 2.68 mM/L, KH_2PO_4 1.47 mM/L, NaCl 547.5mM/L, $NaHPO_4$ 9.50 mM/L). The suspension media used in the *in vivo* experiments was supplied by Medisorb and consisted of 0.5% w/v sodium carboxymethylcellulose (medium viscosity) and 0.1% w/v Tween 80. A Coulter® Multisizer II, Coulter Electronics Ltd., Luton, England was used to determine the mass median diameter of the microspheres.

Polymer synthesis and Local Anesthetic Incorporation.

The radiolabeled microspheres were formulated by a single emulsion technique, using an evaporation process. Two types of radiolabeled microspheres were formulated, one which contained 75% w/w unlabeled bupivacaine and 0.05% w/w tritium labeled dexamethasone and the other contained 0.05% w/w unlabeled dexamethasone and 75% w/w tritium labeled bupivacaine. The microspheres which contained tritium labeled dexamethasone were prepared as follows: an aliquot of dexamethasone containing 8 x 10^6 disintegrations per minute (dpm) was added to 100 μls of a solution of 5 mg of unlabeled dexamethasone in 5 mls of ethanol. The sample was dried under a stream of nitrogen for one hour and 50 mg of PLGA 65:35 and 150 mg of bupivacaine free base in 1 ml of CH_2CL_2 were added. The tube was vortexed for 1 minute at 2000 rpm on a Fisher Scientific Touch Mixer, Model 232. The 1 ml of 0.3% polyvinylalcohol in 100 mM Trisma® (tris(hydroxymethyl)amino methane) base (pH adjusted to 8.4) was added, and an emulsion formed by vortexing for 45 seconds. The emulsion was then poured into 100 mls of 0.1% polyvinylacohol in 100 mM Trisma® base. The CH_2CL_2 was removed from the microspheres using a rotary evaporator under vacuum at 40OC for 20 minutes. After 2-3 minutes bubbles

formed indicated that the organic solvent was being removed. The microspheres were sieved through a series of stainless steel sieves of pore sizes 140 μ, 60 μ and 20 μ (Neward Wire Co.). Those microspheres which were less than 20 and greater than 140 microns in diameter were discarded. The microspheres which fell in the size range 20 μ to 140 μ were centrifuged at 4000 rpm for 5 minutes, rinsed with buffer and centrifuged again. The microspheres were then frozen in liquid nitrogen and lyophilized overnight. The microspheres were examined before and after solvent removal using an American Opitcal One - Ten light microscope to ensure that no leaching of the drug took place. If leaching did occur, the bupivacaine crystallized and could be seen even at 10X using a light microscope.

The microspheres which contained tritium labeled bupivacaine were formulated as described above with the following exceptions: An aliquot of radiolabeled bupivacaine consisting of 9 x 10^6 dpm was added to 150 mg of unlabeled bupivacaine free base. The solution was then vortexed to ensure homogeneous mixing of labeled and unlabeled bupivacaine. The ethanol was then removed under a stream of nitrogen for 1 hour. Upon removal of the ethanol, 50 mg of PLGA 65:35 and 100 μl from a solution dexamethasone 1 mg/ml in ethanol were added. Thereafter, the protocol was the same as that used to formulate microspheres which contained radiolabeled dexamethasone.

In order to determine the drug content, 5 mg of microspheres were dissolved in 2 mls of CH_2Cl_2 and the local anesthetic concentration determined by U.V. spectroscopy. The absorbance at 272 nm was read and compared to a calibration curve of

known amounts (0 to 2.5 mg/ml) of bupivacaine free base dissolved in CH_2Cl_2.

<u>*In Vitro* Release studies</u>

Unlabeled Microspheres

5 mg of microspheres were weighed out and 2 mls of Dulbecco's phosphate-buffered saline was added. The pH of the buffer was adjusted to 7.4 and 0.1% sodium azide was added as an antimicrobial agent. The buffer was changed at 0.5, 2, 6, 12, and 24 hours and once daily thereafter. The amount of bupivacaine free base in the buffer was determined using a Hewlett Packard 8452 Diode Array Spectrophotometer at 272 nm. Duplicates fron each batch of microspheres were assayed. Release media incubated with control microspheres which did not contain bupivacaine showed insignificant absorbance at 272 nm.

<u>Labeled Microspheres</u>

The procedure used to determine the *in vitro* release of both bupivacaine and dexamethasone is the same as that used for non-radiolabeled microspheres, except that the amount of radiolabeled compound released into the buffer was determined by adding 17 mls of Ecolume® scintillation fluid to 2 mls of buffer. The total number of counts was determined using a LKB Wallac 1214 Rackbeta Liquid Scintillation Counter. The efficiency, (the counts per minute/disintegration per minute), of the counter was determined to be 51%. Five replications of each set of radiolabeled microspheres were used.

<u>Preparation of Microsphere Suspensions for *In Vivo* Testing</u>

The dose used varied between 50 and 450 mg of drug/kg of rat, and 0.6 mls of injection vehicle was used for each injection. The injection vehicle consisted of 0.5% w/w sodium carboxy methyl

cellulose and 0.1% w/w Tween 80 in water. The microspheres in the suspending media were vortexed at maxium speed for two minutes prior to injection. The injection was performed by locating and injecting slightly below and proximal to the greater trochanter. Rats were anesthetized with halothane 2-3% inspired concentration in oxygen during injections, at least five rats were used to test each formulation.

Testing for Sciatic Nerve Block

Male Sprague-Dawley Charles River rats weighing between 200 and 350 mg were used to determine the duration of the block obtained with each of the different microsphere formulations tested. They were handled daily and habituated to the testing paradigm prior to exposure to local anesthetic injections. Sensory and motor blockade were examined as described above. The duration of the sensory block was determined as the length of time for which the latency was greater than or equal to 7 seconds.

In addition to sensory testing, motor testing was performed at each time point to examine the rat's ability to hop and to place weight on its hind leg. Animals were handled and cared for according to institutional, state, and federal regulation, and according to the guidelines of the International Associationi for the Study of Pain, Seattle, Washington.

Results

Microsphere morphology

Using the preparative procedures outlined above, smooth, spherical, mechanically stable microspheres were produced without significant quantities of crystalline bupivacaine leaching out the microspheres. When the drug leached out of the microspheres into the aqueous solution, it was in

the form of long crystals, approximately 30 μ in length and was visible by light microscopy. Comparison of PLGA microspheres loaded with 75% bupivacaine and 0.05% dexamethasone formulated by solvent removal using a vaccum at 40°C with those formulated by stirring the microspheres at room temperature and pressure, for three hours until the organic solvent evaporated, showed no differences. Increasing the rate of removal of the organic solvent using heat and vaccum reduced the rate of leaching of bupivacaine out of the microspheres from 40% to 2%.

In vitro release kinetics

Figure 1 is a graph of % cumulative release from PLA and PLGA copolymers, PL:GA 50:50, 75:25, and 65:35, over time. The results demonstrate that there is a burst of release from PLA initially, which is substantially less in the PLGA copolymers.

Other polymers have been used to achieve similar results. Ethyl cellulose and polyhydroxyvalerate-butyrate (75:25) microspheres (20 to 140 microns in diameter) containing 50 and 75% by weight bupivacaine, with or without 0.05% dexamethasone, showed 30 to 50% release by day 3 *in vitro*, with efficacy in *in vivo* studies.

The similar *in vitro* release rates of bupivacaine from PLGA 50:50, 65:35, 75:25 PLGA and PLA are shown in Figure 2. Comparison of the % cumulative release of bupivacaine from microspheres when the pH of the buffer media was 6,7.4 and 8 shows that the rate of release of bupivacaine was higher at pH 6 than at pH 7.4 or 8, because bupivacaine has greater water solubility at pH 6 than at pH 7.4 or pH 8.

Radiolabeled microspheres

When microspheres loaded with unlabeled bupivacaine and radiolabled dexamethasone were

prepared, the yield (weight of microspheres/weight of bupivacaine + weight of polymer) was 45%. The bupivacaine content was determined to be 75 ± 1%. When microspheres loaded with unlabeled dexamethasone and radiolabeled bupivacaine were prepared, the yield was 50%, and the bupivacaine content was 73 ± 2%. Comparisons of the percent cumulative release of both tritum labeled dexamethasone and tritium labeled bupivacaine, proves that dexamethasone was incorporated into the microspheres and that both substances were released at similar release rates. The comparison of the two techniques, U.V. spectroscopy and scintillation counting, used to monitor the *in vitro* release of unlabeled and radiolabeled bupivacaine respectively, show that the same release rate occurred using the two techniques.

Rat Sciatic Nerve Blockade *In Vivo*

In order to determine the toxic response of the rats to various microsphere doses, the rats were injected with concentrations ranging from 50 to 450 mg of drug/kg of rat for each type of polymer. The corresponding plots of duration of block versus concentration polots of duration of block versus concentration of dexamethasone is shown in Figures 3a and 3b. No systemic toxicity, excessive sluggishness or death was observed even at the highest doses.

A comparison of the latencies and mean motor times obtained from PLGA 65:35 microspheres which contained 0%, 0.005% and 0.05% dexamethasone at a dose of 150 mg/Kg of rat showed duration of the blocks were 8, 50 and 170 hours, and decreased motor skills over 30, 70, and 190 hours,as shown in Figure 3a and Figure 3b, respectively. The optimum dose and formulation was determined to be 150 mg of drug/kg of rat of PLGA 65:35 microspheres loaded

with 75% bupivacaine and 0.05% dexamethasone, as this was the lowest dose which resulted in the longest duration of block.

The presence of 0.05% dexamethasone in microspheres significantly prolonged the duration of sciatic nerve block. That is, the block obtained using microspheres which contained 0.05% dexamethasone was up to 13 fold longer than the block obtained using the corresponding microspheres which did not contain any dexamethasone. It was determined that 150 mg of microspheres/Kg of rat was the optimum dosage and any further prolongation of block obtained by using a higher dose of injecting a higher dose. The optimum dose and formulation was determined to be 150 mg of drug/Kg of rat of PLGA 65:35 microspheres which contained 75% bupivacaine and 0.05% dexamethasone (the mass median diameter was 70 μ, determined using a coulter counter). Using this formulation a 134 hour sciatic nerve block was achieved.

Figures 4a-d compare the duration of sensory block for groups of rats injectd with bupivacaine loaded PLA 100, PLGA 75:25, PLGA 65:35 and PLGA 50:50 microspheres, with and without incorporated dexamethasone. In each case, the presence of dexamethasone in the microspheres resulted in a 6-13 fold increase in the duration of block. Mean sciatic nerve block durations among treatment groups varied from 65 $\pm$ 3 to 134 $\pm$ 13 hours for microsphere formulations which contained dexamethasone. Control groups receiving injections of polymer microspheres containing no drug or dexamethasone or containing dexamethasone alone showed no sensory or motor block.

The *in vitro* results showed that the bupivacaine was released from the microspheres in a controlled manner. In general, 24-40% of the

bupivacaine was released in the first 24 hours, and approximately 7% released daily thereafter. After 5-8 days approximately 90% of the bupivacaine was released. The presence of dexamethasone in the microspheres did not significantly affect the *in vitro* release rates of bupivacaine and the *in vitro* results cannot account for the prolongation of block, due to the presence of dexamethasone observed *in vivo*.

Example 2: Administration of Microspheres in combination with Glucocorticoids in solution.

Example 1 demonstrated that incorporation of 0.05% dexamethasone into either pellets or microspheres resulted in prolongation of block, from 70-100 hours when microspheres which contained 0.05% dexamethasone were used versus 50-60 hours in the case of microspheres which contained no dexamethasone. To further understand the mechanism, a model system was developed whereby different additives: steroids, steroidal anti-inflammatories, and non-steroidal antiinflammatories (NSAIDs), were placed in the injection fluid to determine if the block could be prolonged. In this model system, the additives were placed into the injection fluid immediately prior to injection, and the microspheres used contained bupivacaine, but no dexamethasone. If the additive was a solid, it was dissolved in ethanol and aliquots of concentrations which varied between 0.005 and 5% w/w added. If the additive was in liquid form, then the amount was added directly to the injection fluid.

The results demonstrate that (1) Bupivacaine-polyester microspheres can produce sciatic nerve blockade with a wide margin of safety with regard to systemic toxicity.

(5) The duration of sciatic blockade from bupivacaine-polyester microspheres is prolonged by incorporation of glucocorticoid into the microspheres, which is proportional to the strength of the glucocorticoid.

Materials and Methods

Formulation of PLGA Microspheres and Protocol for *In Vitro* Release Studies

Formulation of Microspheres of 65:35 loaded with 75% bupivacaine with 0.05% dexamethasone.

50 mg of PLGA 65:35 (High molecular weight, purchased from Medisorb) and 150 mg of bupivacaine free base (obtained from Perdue-Frederick) were dissolved in 0.1 ml of a solution of 5 mg of dexamethasone in 5 mls in CH_2CL_2 and 0.9 mls of CH_2CL_1. 1 ml of 0.3% polyvinyl alcohol (PVA) in 100 mM Tris buffer at pH 8.5 was added and the mixture vortexed 3 times for 15 seconds each time. The mixture was poured into 100 mls of 0.1% PVA in 100 mM Tris buffer. The microspheres were examined using the light microscope and the size distribution was determined to be between 10 and 110 microns. The CH_2Cl_2 was removed by heating the sample to 45°C using a rotary evaporator at full vacuum for 15 minutes. The suspension of microspheres in 0.1% PVA was filtered through 140, 60, and 20 μ metal sleeves (Newark Wire Cloth Co.). Then the microspheres were frozen in liquid nitrogen and lyophilized overnight.

Formulation of Microspheres which contained tritium labeled dexamethasone

Radiolabeled dexamethasone was purchased from Amersham and an aliquot which contained 200,000 counts was added to cold dexamethasone and the microspheres were formulated as above.

Formulation of Microspheres which contained tritium labeled Bupivacaine

Radiolabeled bupivacaine was kindly donated by Dr. Gary Strichard from Brigham and Woman's Hospitl. Again the bupivacaine was dissolved in ethanol and an aliquot which contained 200,000 counts was added to cold bupivacaine and the microspheres were formulated as above.

Analysis of the *in vitro* release of either tritium labeled dexamethasone or bupivacaine

The *in vitro* release studies were carried out as outlined above except that instead of monitoring the release by U.V. spectroscopy, the *in vitro* release was determined by adding 15 mls of Ecolume™ to each 2 ml aliquot of buffer, and the subsequent disintegrations were monitored using a scintillation counter.

Preparation of the Suspension

A ratio of 150 mg bupivacaine/kg was injected. The corresponding amount of microspheres is 200 mg/kg. The microspheres are weighed out and transferred to a 3 cc syringe via the plunger. The needle of the syringe is removed and the opening covered with parafilm. Carboxymethylcellulose sterilized by filtration through a 0.2 micron filter is used as the injection fluid.

The rats are tested at 0.5, 1, 2, 3, 6, 8 and 24 hours after injection and then once daily until the block wears off. The rat is motor and sensory tested each time as described above using a hotplate at 56°C.

Results

The results are shown in Table 2.

Table 2: PLGA Polymer 150 mg/kg + Additives

# of rats	Additives (Conc)	Class of Additive	Duration of block (hrs)
7	Dexamethasone (0.05%)	Anti-Inflammatory/ Steroid (strong)	50-60
5	Dexamethasone (.005%)	Anti-Inflammatory/ Steriod (strong)	5-6
5	Dexamethasone (.5%)	Anti-Inflammatory/ Steriod (strong)	24 *2 Rats Died 2 Weeks Later
8	Cholesterol (0.05%)	Steroid	3-4
5	Cholesterol (0.5%)	Steroid	5
5	Epinephrine (.05%)	Cardiovascular Drug	6-7* Rats Became Sick
5	Ketorolac (0.5%)	Anti-Inflammatory (strong)	6-7
5	Ketorolac (5%)	Anti-Inflammatory (strong)	6-7
5	Methyl-prednisolone (5%)	Anti-Inflammatory/ Steroid (medium)	20

7	Methyl-predoisolone (0.5%)	Anti-Inflammatory/ Steriod (medium)	25
5	Estradiol (0.5%)	Steroids	6-8
4	Estradiol (0.05%)	Steroid	7
71	Hydrocortisone (0.5%)	Anti-Inflam/Steroi d (weak)	8-9
5	Hydrocortisone (5%)	Anti-Inflam/Sterio d (weak)	13
5	Testosterone (.05%)	Steroid	10-15
5	Betamethasone (.05%)	Anti-Inflam/Steroi d (strong)	40-45

The results comparing sensory and motor block following administration of dexamethasone in the injection fluid with dexamethasone in the microspheres is shown in Figures 5a and 5b.

The results demonstrate that dexamethasone does not produce sciatic blockade by itself in solution, nor does it prolong blockade from bupivacaine in solution. Addition of dexamethasone in solution with bupivacaine in solution did not prolong blockade relative to bupivacaine in solution alone. The prolonged blockade previously observed seemed to require the presence of bupivacaine in microspheres.

A model system was developed in which dexamethasone was dissolved in ethanol and an aliquot of known concentration was added to the suspending medium which contained microspheres loaded with 75% bupiviaine. Addition of dexamethasone to the suspending medium in concentrations ranging from 0.05% to 0.5% prolonged the duration of blockade obtained using bupivacaine microspheres. Addition of 0.005% w/w bupivacaine did not result in a prolongation of the blockade obtained. The result of this model system was useful, because it permitted testing of a series of compounds over full concentration ranges for prolongation of sciatic block *in vivo* without the labor-intensive step of making a microsphere prep with each additive and each dose.

Studies were conducted to determine whether dexamethasone's prolongation of blockade is unique, or whether it can be replicated by: (1) other glucocorticoids, (2) other classes of steroids, or (3) other drugs with anti-inflammatory activity, including non-steroidals (NSAIDs). For example, it is well known that cholesterol and other steroids modify membrane lipid phase equilibria, and it is conceivable that effects on lipid physical state could perturb sodium channel function and amplify or prolong channel blockade from local anesthetics. The question was also raised as to whether the dexamethasone effect was due to changes in regional perfusion, analogous to epinephrine's effect.

Table 1 summarizes the results of these experiments. Figure 6 compares the effect of various glucocorticoids on duration of nerve blockade when administered in combination with microspheres having bupivicaine incorporated therein. It can be seen that:

1. High potency glucocorticoids such as betamethasone also produce

prolongation of block up to 45 hours in duration.

2. Intermediate potency glucocorticoids such as methylprednisolone produce intermediate degrees of block prolongation.
3. Weaker glucocorticoids such as hydrocortisone produce mild, but statistically significant prolongation of block.
4. The weaker prolongation of block by hydrocortisone cannot be made as effective as dexamethasone by further increasing its concentration in the suspending medium.
5. Estrogen have no block-prolonging effect. Testosterone may have shown mild prolongation of blockade.
6. NSAIDs and epinephrine did not substantially prolong blockade. Epinephrine in the doses used (0.05% in the suspending medium) produced considerable systemic toxicity, but no deaths.

Preliminary reports on the histologic effects are that they are benign, with no evidence of major axonal or demyelinating injury and only mild inflammation.

A long duration of block was produced using 150 mg/kg rat body weight with 75% bupivacaine loaded PLGA 65:35 microspheres. Doses as high as 600 mg/kg can be given with temporary somnolence as a side-effect, but no convulsions or cardiac arrests.

The dosing of dexamethasone in the microspheres (0.05%) is quite low, particularly considering its delayed release. Even when this

concentration of dexamethasone was added in the suspending medium (permitting immediate access for absorption), no systemic effects were found. In one experiment using dexamethasone 0.5% in the suspending medium, no immediate toxicities occurred, but among five rats there were two deaths at 12-15 days post injection, and at the same time a third rat appeared thin and pale.

Experiments confirmed that 65:35 PLGA polymers were preferable to either 75:25 PLGA or 100% PLA, both in terms of (1) the reliability, intensity and duration of sciatic nerve block, (2) each of dispersal and injectability. A blockade of 30-40 hours was observed with PLGA 50:50 over the PLGA 65:35 microspheres, indicating no advantage.

Example 3: The combination of local anesthetic in microspheres with glucocorticoid is not a result of altered release rates *in vivo*.

Additional studies were conducted as described above to further elucidate the mechanisms involved in the prolongation of the nerve blockade by the glucocorticoid.

Figure 7 is a graph of the amount of bupivacaine (weight mg) in microspheres extracted from rats as a function of time in days following injection. The study compared the amount of bupivacaine released as a function of polymer, comparing PLGA 75:25 with and without dexamethasone, PLGA 65:35 with and without dexamethasone, PLGA 50:50 with and without dexamethasone, and PLA containing bupivacaine and dexamethasone. The results demonstrate that the drug is being released over time as expected, and that release is not altered by the presence or absence of dexamethasone. Accordingly, the glucocorticoid must be exerting an effect directly on the nerve, not by interaction with the local anesthetic.

We claim:

1. A method for prolonging the effect of a local anesthetic administered at a site in a patient comprising

administering at the site a formulation comprising a local anesthetic in a controlled release form and a glucocorticoid in an amount effective to prolong the effect of the local anesthetic.

2. The method of claim 1 wherein the controlled release form is a polymer in the form slabs, beads, pellets, microparticles, microspheres, microcapsules, pastes or suspensions.

3. The method of claim 2 wherein the polymer is selected from the group consisting of polyanhydrides, poly(hydroxy acids), polyorthoesters, proteins, and polysaccharides.

4. The method of claim 3 wherein the polymer is a copolymer of lactic acid and glycolic acid.

5. The method of claim 1 wherein the controlled release form is liposomes, lecithin microdroplets, polymer-lipid microparticles.

6. The method of claim 1 wherein the anesthetic is bupivacaine, dibucaine, etidocaine, ropivacaine, tetracaine, lidocaine, xylocaine, or salts and mixtures thereof.

7. The method of claim 1 wherein the glucocorticoid is dexamethasone, cortisone, hydrocortisone, prednisone, beclomethasone, dipropianate, betamethasone, flunisolide, methyl prednisone, para methasone, prednisolone, triamcinolome, alclometasone, amcinonide, clobetasol, fludrocortisone, diflurosone diacetate, fluocinolone acetonide, fluoromethalone, flurandrenolide, halcinonide, medrysone,

mometasone, or pharmaceutically acceptable salts and mixtures thereof.

8. The method of claim 1 wherein the glucocorticoid is administered in a solution with the controlled release form.

9. The method of claim 1 wherein the formulation is administered by injection.

10. A formulation for prolonging the effects of local anesthesia at a site in a patient comprising

a local anesthetic in a controlled release form and

a glucocorticoid effective in an amount effect to prolong the effect of the local anesthetic.

11. The formulation of claim 10 wherein the glucocorticoid is incorporated into the controlled release form with the local anesthetic.

12. The formulation of claim 10 wherein the glucocorticoid is in solution for administration with the controlled release form.

13. The formulation of claim 10 wherein the anesthetic is incorporated into a polymer.

14. The formulation of claim 13 wherein the polymer is selected from the group consisting of polyanhydrides, poly(hydroxy acids), polyorthoesters, proteins, and polysaccharides.

15. The formulation of claim 14 wherein the polymer is a copolymer of glycolic acid and lactic acid.

16. The formulation of claim 10 wherein the controlled release form is liposomes, lecithin microdroplets, polymer-lipid microparticles.

17. The formulation of claim 10 wherein the anesthetic is selected from the group consisting of bupivacaine, dibucaine, etidocaine,

tetracaine, ropivacaine, lidocaine, xylocaine and salts and mixtures thereof.

18. The formulation of claim 10 wherein the anesthetic is incorporated into the controlled release form at a percent loading of between 0.1% and 90%.

19. The formulation of claim 10 wherein the glucocorticoid is incorporated into the controlled release form at a percent loading of between 0.01 and 30% by weight.

20. The formulation of claim 10 wherein the glucocorticoid is selected from the group consisting of dexamethasone, cortisone, hydrocortisone, prednisone, beclomethasone, dipropianate, betamethasone, flunisolide, methyl prednisone, para methasone, prednisolone, triamcinolome, alclometasone, amcinonide, clobetasol, fludrocortisone, diflurosone diacetate, fluocinolone acetonide, fluoromethalone, flurandrenolide, halcinonide, medrysone, mometasone, and pharmaceutically acceptable salts and mixtures thereof.

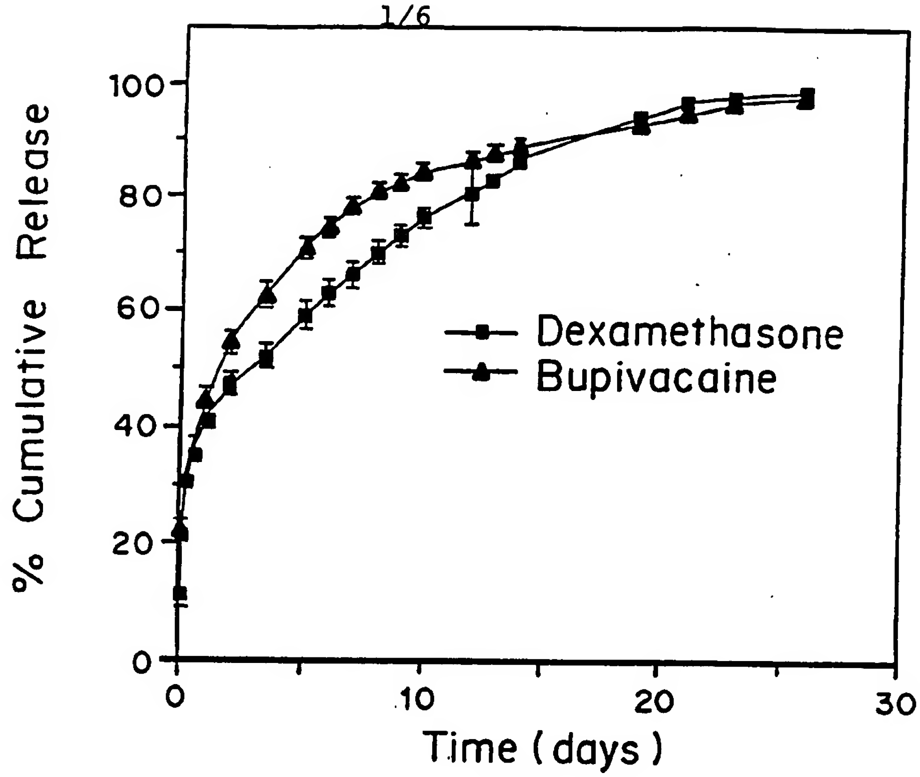

% Cumulative Release
100
80
60
40
20
0
0
10
20
30
Time (days)
Dexamethasone
Bupivacaine

FIG. 1

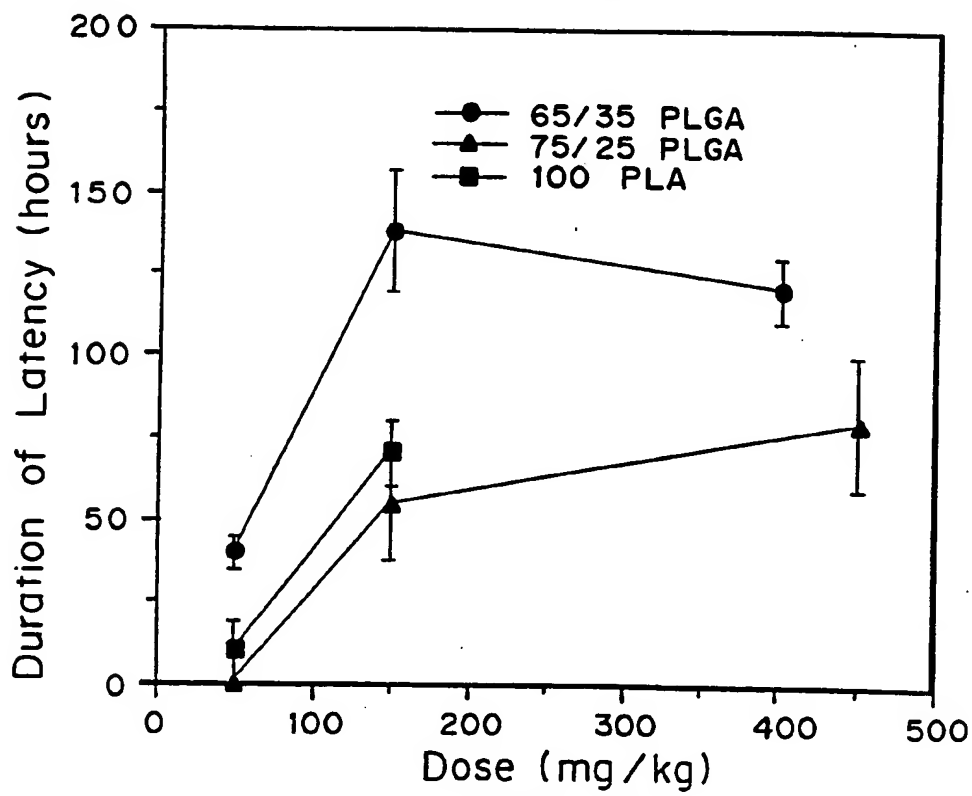
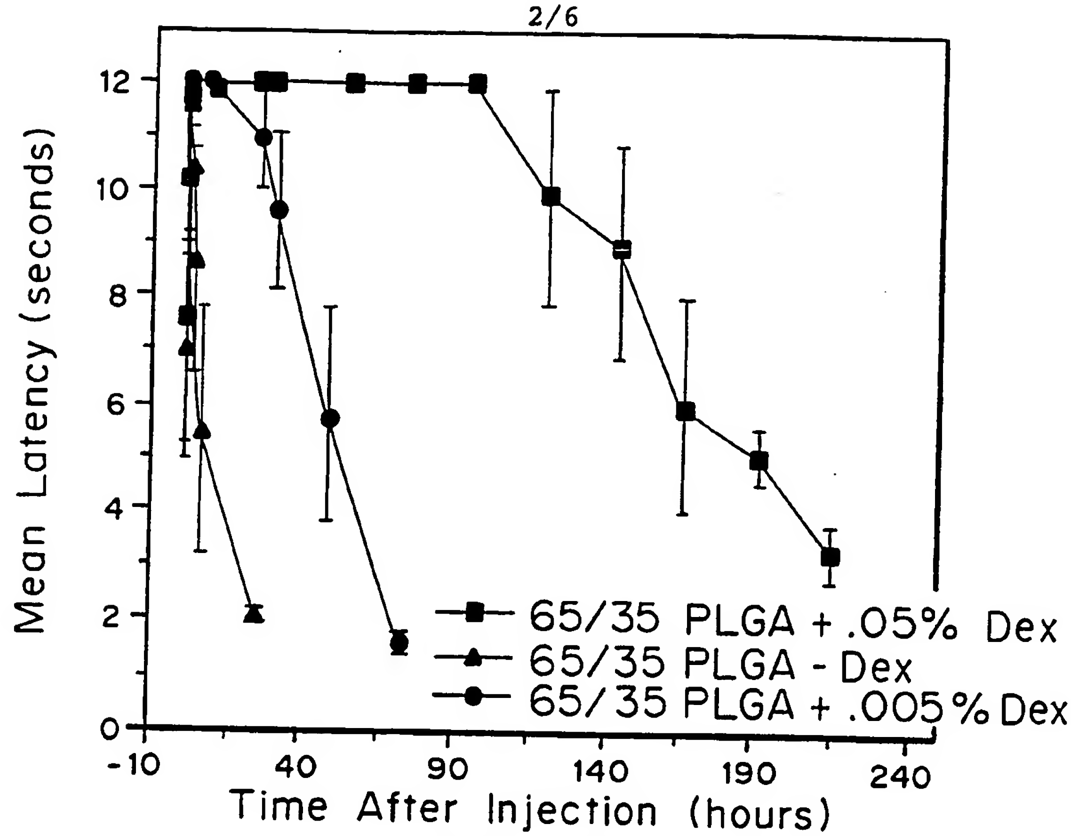
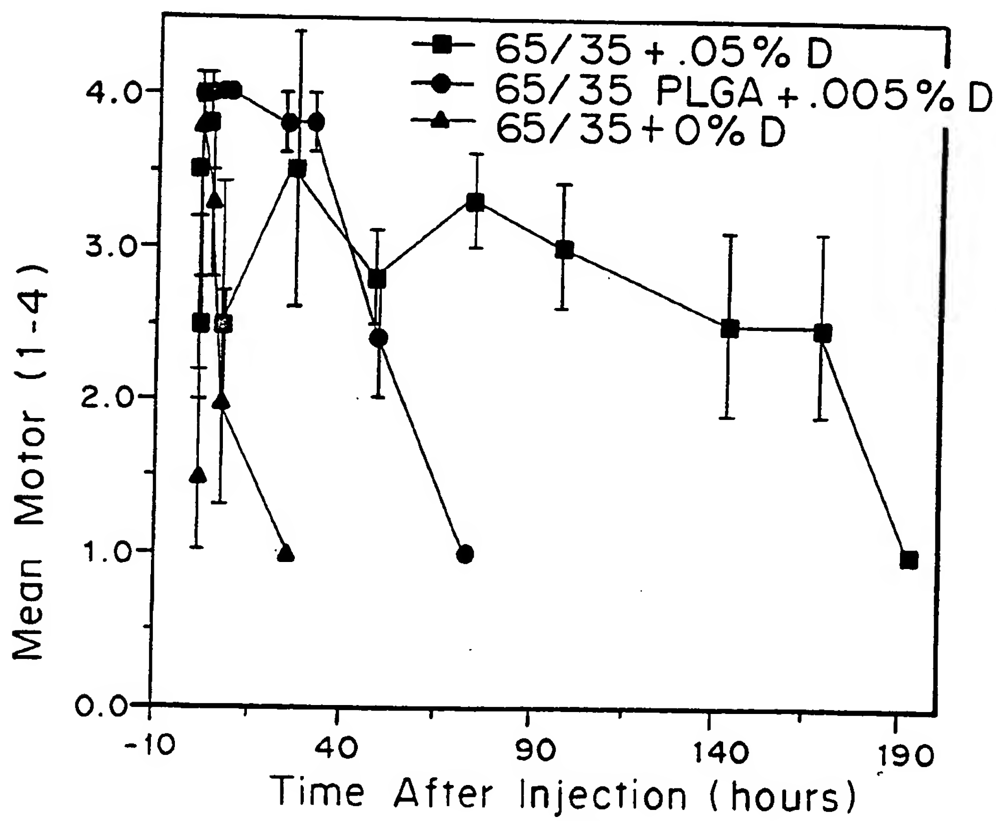

Duration of Latency (hours)
200
150
100
50
0
0
100
200
300
400
500
Dose (mg/kg)
65/35 PLGA
75/25 PLGA
100 PLA

FIG. 2



Mean Latency (seconds)
0
2
4
6
8
10
12
-10
40
90
140
190
240
Time After Injection (hours)
65/35 PLGA + .05% Dex
65/35 PLGA - Dex
65/35 PLGA + .005% Dex

FIG. 3a



Mean Motor (1-4)
0.0
1.0
2.0
3.0
4.0
-10
40
90
140
190
Time After Injection (hours)
65/35 + .05% D
65/35 PLGA + .005% D
65/35 + 0% D

FIG. 3b

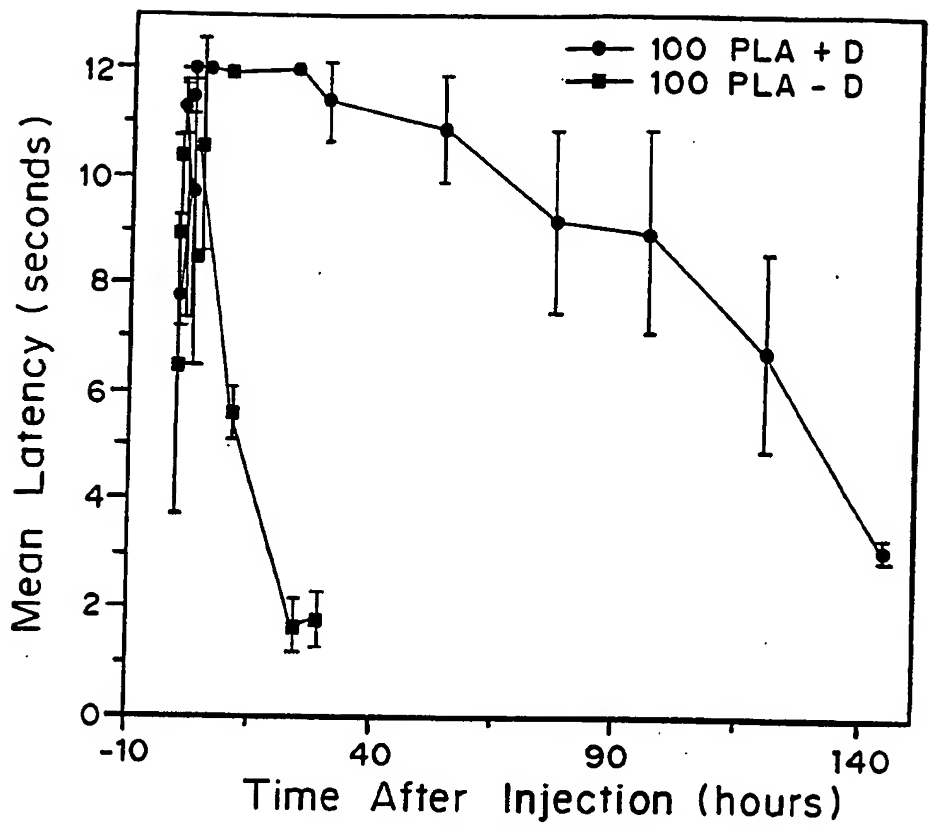

100 PLA + D
100 PLA - D
Mean Latency (seconds)
0
2
4
6
8
10
12
-10
40
90
140
Time After Injection (hours)

FIG. 4a

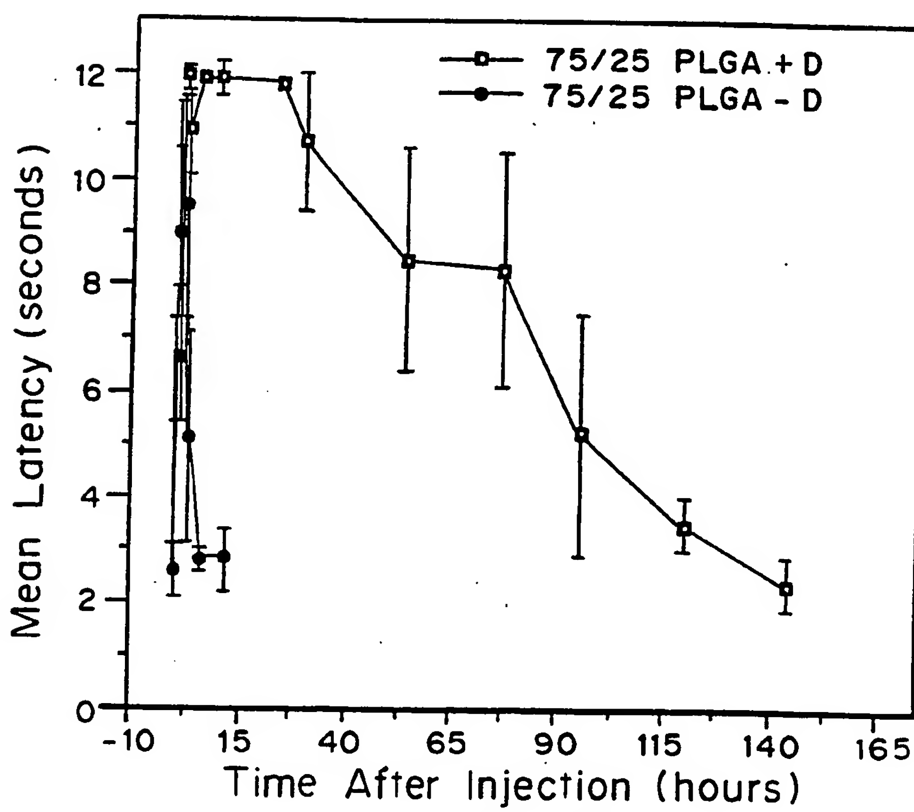

75/25 PLGA + D
75/25 PLGA - D
Mean Latency (seconds)
0
2
4
6
8
10
12
-10
15
40
65
90
115
140
165
Time After Injection (hours)

FIG. 4b

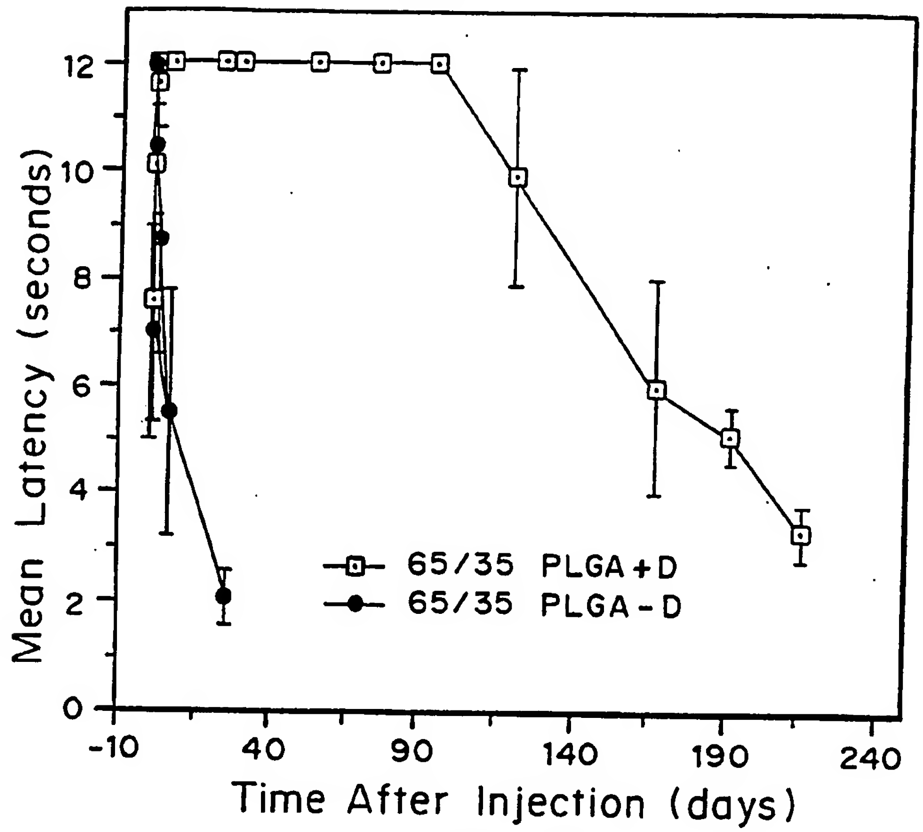

Mean Latency (seconds)
0
2
4
6
8
10
12
-10
40
90
140
190
240
Time After Injection (days)
65/35 PLGA+D
65/35 PLGA-D

FIG. 4c

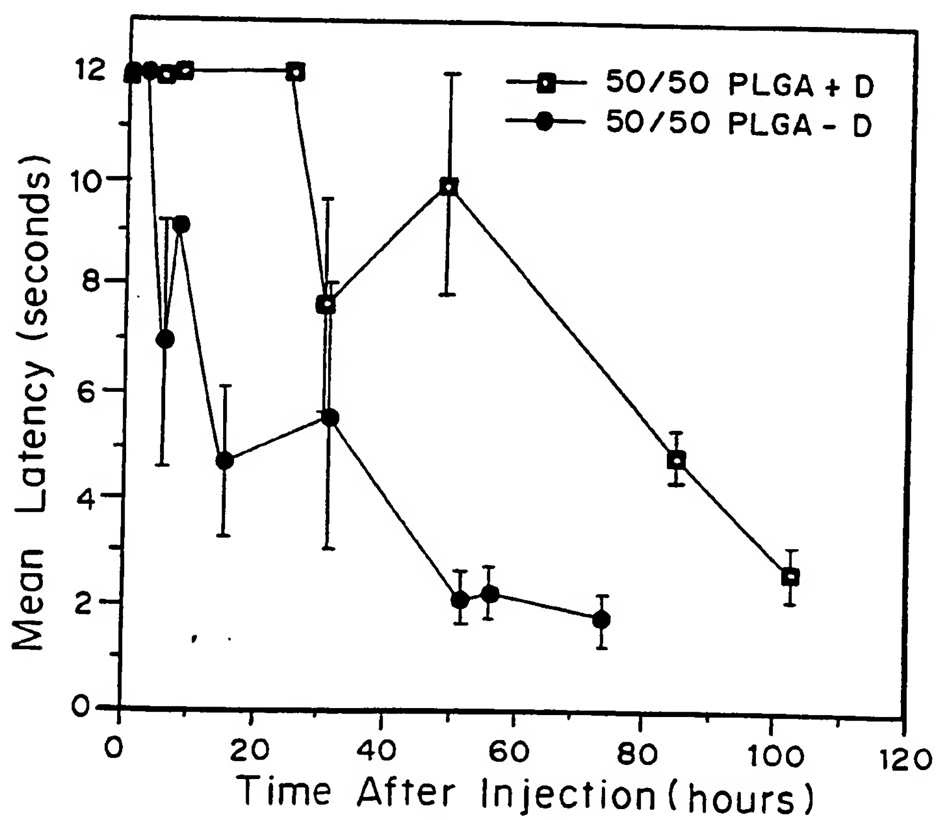

Mean Latency (seconds)
0
2
4
6
8
10
12
0
20
40
60
80
100
120
Time After Injection (hours)
50/50 PLGA + D
50/50 PLGA - D

FIG. 4d

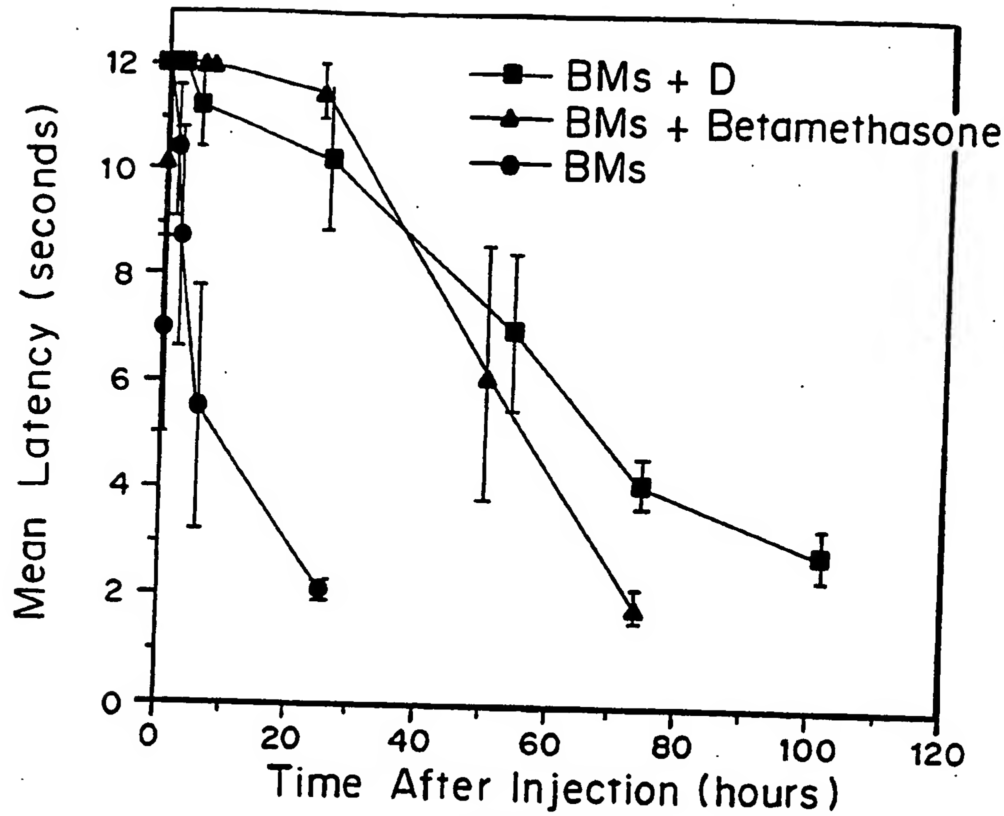

BMs + D
BMs + Betamethasone
BMs
Mean Latency (seconds)
Time After Injection (hours)
0
2
4
6
8
10
12
20
40
60
80
100
120

FIG. 5a

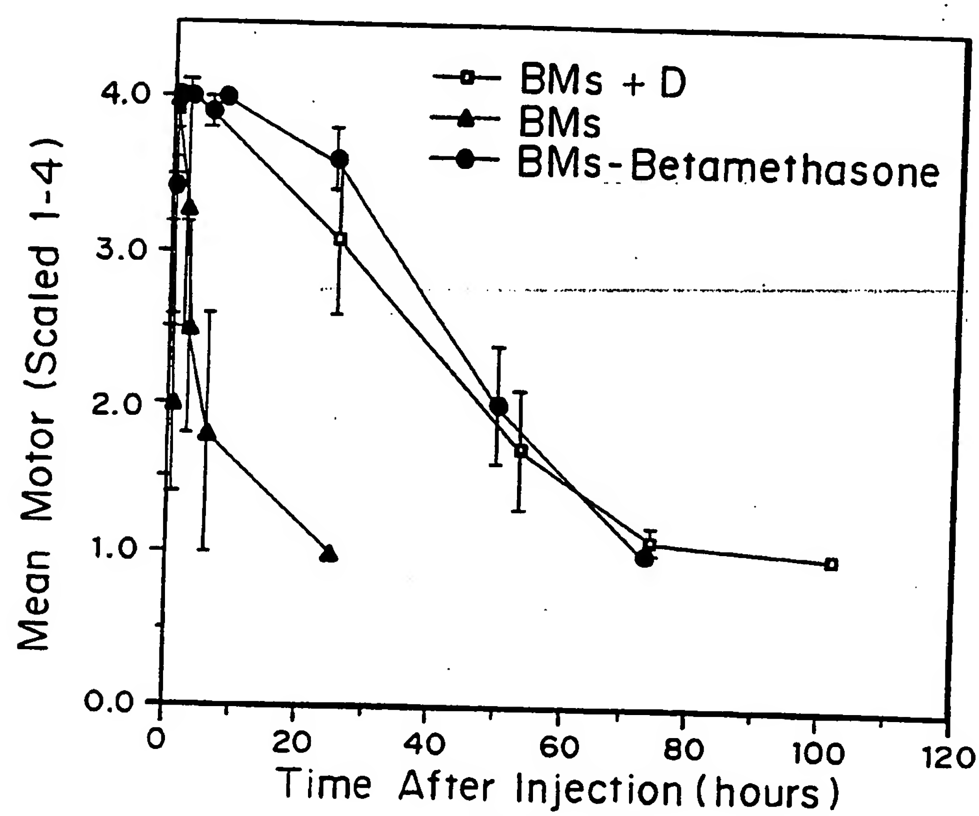

BMs + D
BMs
BMs-Betamethasone
Mean Motor (Scaled 1-4)
Time After Injection (hours)
0.0
1.0
2.0
3.0
4.0
0
20
40
60
80
100
120

FIG. 5b

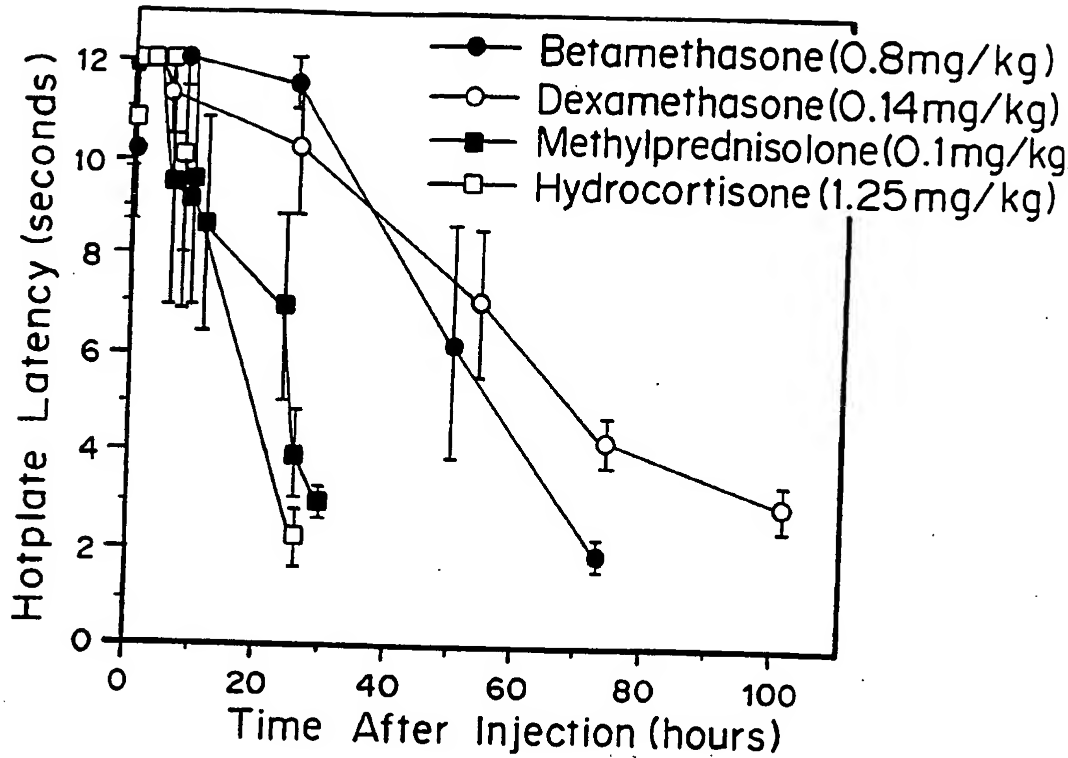

Betamethasone(0.8mg/kg)
Dexamethasone(0.14mg/kg)
Methylprednisolone(0.1mg/kg)
Hydrocortisone(1.25mg/kg)
Hotplate Latency (seconds)
0
2
4
6
8
10
12
0
20
40
60
80
100
Time After Injection (hours)

FIG. 6

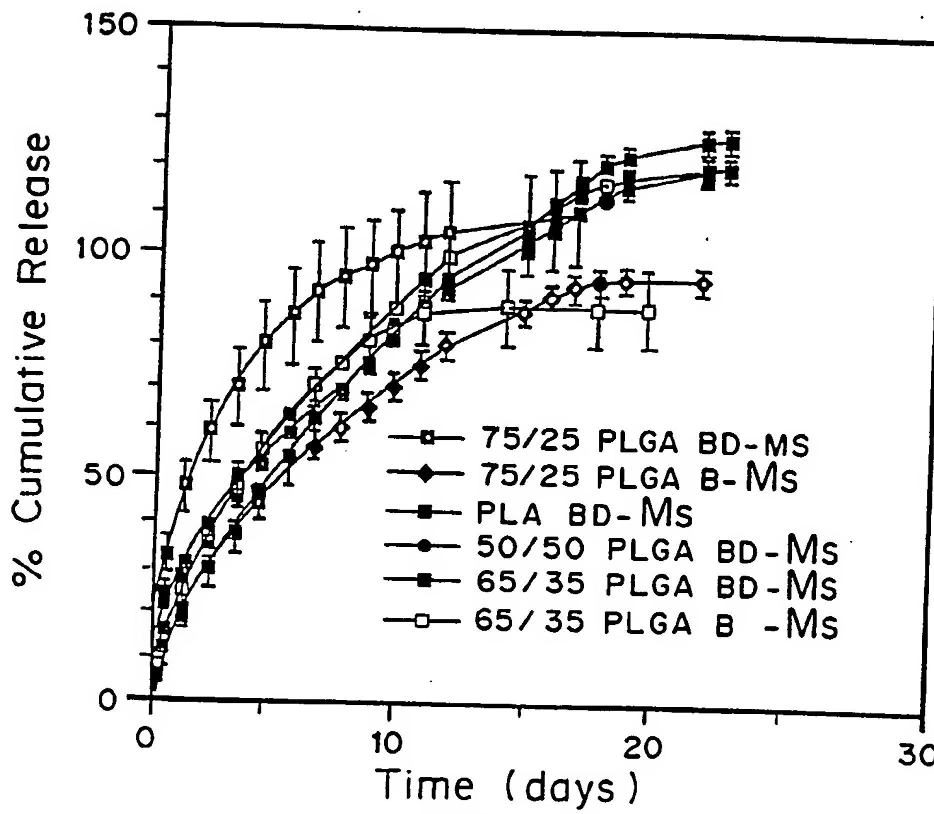

% Cumulative Release
0
50
100
150
0
10
20
30
Time (days)
75/25 PLGA BD-MS
75/25 PLGA B-Ms
PLA BD-Ms
50/50 PLGA BD-Ms
65/35 PLGA BD-Ms
65/35 PLGA B -Ms

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/06085

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/16 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 05265 (CHILDREN'S MEDICAL CENTER CORPORATION) 17 March 1994 cited in the application see the whole document -----	1-4, 6-15, 17-20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 1996	16.09.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ventura Amat, A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06085

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-9 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/06085

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9405265	17-03-94	AU-B- 5126993	29-03-94
		CA-A- 2144407	17-03-94
		EP-A- 0659073	28-06-95
		JP-T- 8503695	23-04-96